**Amendments to the Specification**

Please replace the paragraph that begins at page 65 with the following:

It has been shown that human chromosome 14 fragment SC20 (deposited as Accession Number FERM BP-7583 in the International Patent Organism Depository on May 9, 2001), is retained almost 100% in a mouse and can be transmitted to progeny. Therefore, in this invention, SC20 is used as a human artificial chromosome vector. That is, the translocation of HCF2-Igλ-LIF fragment of human chromosome 22 and cosYHZ304-Igκ-CD8A fragment of human chromosome 2 as described above in A and B to SC20 resulting in construction of human artificial chromosomes λ and κ-HAC having the fragments of the human chromosomes 22 and 2 cloned thereto.

Please replace the paragraph that bridges pages 1 and 2 with the following paragraph:

The present invention relates to pluripotent cells in which endogenous genes are disrupted, use of the same, and a method for producing chimeric non-human animals by microcell method and use of the animals. If a foreign chromosome or a fragment thereof containing a gene encoding a gene product identical with or homologous to the gene product encoded by the disrupted endogenous gene is transferred into the pluripotent cell of the present invention as a recipient cell so that a desired functional cell or a desired chimeric non-human animal is produced from the cell, the transferred gene can be expressed efficiently without differentiation of the pluripotent cell into a germ cell. Even if a germ cell of the non-human animal is affected or the pluripotent cell cannot be differentiated into a germ cell by the disruption of the endogenous gene or the introduction of a foreign gene, a functional cell, or a chimeric non-human animal, a tissue or a cell of the animal can retain and express a foreign giant DNA fragment in excess of the heretofore unattainable 1 Mb (a million bases) in conditions of a deficiency in the endogenous gene and a decrease in the production of an endogenous gene product by producing the desired functional cell or non-human animal from the pluripotent cell.

Please replace the paragraph that bridges pages 3 and 4 with the following paragraph:

However, there had been a limit of the size of DNA capable of being transferred and this restricts the application range of these techniques. The limit depends on the size of DNA which can be cloned. One of the largest DNA fragments which have ever been transferred is a DNA fragment of about 670 kb cloned into a yeast artificial chromosome (YAC) (Jakobovits et al., Nature, 362:255, 1993). Recently, introduction of YAC containing an about 1 Mb DNA fragment containing about 80 percent of variable regions and portions of constant regions (Cμ, Cδ and Cγ2) of a human antibody heavy-chain was reported (Mendes et al., Nature Genetics, 15:146, 1997). These experiments were carried out by fusing a YAC-retaining yeast cell with a mouse ES cell. Although it is believed that foreign DNA of up to about 2 Mb can be cloned on YAC (Den Dunnen et al., Hum. Mol. Genet., 1:19, 1992), the recombination between homologous DNA sequences occurs frequently in budding yeast cells and therefore, in some cases, a human DNA fragment containing a large number of repeated sequences is difficult to retain in a complete form. In fact, certain recombinations occur in 20-40% of the clones of YAC libraries containing human genomic DNA (Green et al., Genomics, 11:584, 1991).

Please replace the paragraph that bridges pages 7 and 8 with the following paragraph:

The expectation that a mouse could be generated from a cultured cell turned to a real fact when the ES cell which has stable pluripotency was discovered (Evans et al., Nature, 292:154, 1981). Foreign genes, various mutations and mutations by targeted gene recombination could be introduced into the ES cell, making it possible to perform a wide variety of genetic modifications in mice (e.g., Mansour et al., Nature, 336:348, 1988). The ES cell can be used to produce a mouse having a disrupted target gene by gene targeting techniques. The mouse is mated with a transgenic mouse having a gene of interest to produce a mouse that expresses the gene of interest efficiently. For example, a mouse having a disrupted endogenous antibody gene can be mated with a mouse having a human antibody gene transferred to produce a mouse that expresses the human antibody efficiently. A normal diploid cell has alleles. A transgenic mouse having one allele of an mouse antibody heavy-chain gene disrupted expresses an increased level of human antibody in its serum. A mouse having both alleles of mouse antibody heavy-chain gene disrupted <u>obtained by mating</u> expresses a further remarkably increased level of human antibody (S.D.Wagner et al., Genomics, 35:405-414, 1996).

Please replace the paragraph that bridges pages 8 and 9 with the following paragraph:

Some researchers have developed a technique in which one allele of a target gene is disrupted, and then the concentration of a selective drug is increased, thereby deleting both alleles of the target gene (double knock-out). However, this technique holds the possibility of a decrease in the ability of the target gene-deficient cell to differentiate into a germ cell because the target gene-deficient cell obtained by the high-concentration-selective-culture method is cultured in vivo for a long period and because the drug-selection pressure is severe (Takatsu • Taki, Experimental Medicine, supplement, Biomanual UP Series Basic Techniques for Immunological Study, Yodo-sha, 1995). In another case, if two kinds of selective drugs are used for double knocking-out, for example, if a neomycin-resistant cell is subjected to a double knock-out treatment with hygromycin, the double drug-resistant ES cell is rarely differentiated to produce a mutant mouse (Watanabe et al., Tissue Culture 21, 42-45, 1995). ES cells may lose their differentiation and growth capabilities under certain culture conditions. When a gene targeting procedure is performed twice, ES cells do not lose the ability to differentiate into germ cells of a chimeric mouse but the second homologous recombination frequency is extremely low (Katsuki et al., Experimental Medicine, Vol. 11, No. 20, special number, 1993). Hence, when a target gene-deficient homozygote is produced, particularly when at least two target genes are targeted, a heterozygote mouse deficient in each target gene is produced and then the produced mice are mated with each other to produce a homozygote mouse deficient in at least two genes (N. Longberg et al., Nature, 368:856-859, 1994). If genes to be disrupted exist close to each other and if a mouse deficient in at least two genes cannot be obtained by mating, heterozygote mice deficient in the two target genes are produced from ES cells and they are mated to produce homodeficient mice (J.H. van Ree et al., Hum Mol Genet 4:1403-1409, 1995).

Please replace the paragraph that begins 7 lines from the top of page 15 with the following paragraph:

In the method of item 1 or 2, the foreign chromosome(s) or fragment(s) thereof may be larger than 670 kb, further, at least 1 Mb (one million base pairs). The foreign chromosome or fragment thereof may contain a region encoding an antibody. The microcell containing a foreign chromosome(s) or a fragment(s) thereof may be induced from a hybrid

cell prepared by the fusion of a cell from which the foreign chromosome(s) or fragment(s) thereof is(are) derived, with a cell having a high ability to form a microcell. The microcell containing a foreign chromosome(s) or a fragment(s) thereof may be induced from a cell prepared by a further fusion of the microcell induced from the hybrid cell with a cell having a high ability to form a microcell. The cell from which containing the foreign chromosome(s) or fragment(s) thereof is(are) derived may be a human normal diploid cell. The cell having a high ability to form a microcell may be a mouse A9 cell. The pluripotent cell can be selected from embryonal carcinoma cells, embryonic stem cells, embryonic germ cells and mutants thereof. It is preferred that the foreign chromosome or fragment thereof contains a gene of interest and that the pluripotent cell has a disrupted endogenous gene identical with or homologous to said gene of interest on the foreign chromosome or fragment thereof. It is also preferred that the foreign chromosome or fragment thereof contains at least two genes of interest and that the pluripotent cell has disrupted endogenous genes identical with or homologous to said genes of interest on the foreign chromosome or fragment thereof. In the pluripotent cell, one or both alleles of an endogenous gene identical with or homologous to the gene of interest on the foreign chromosome or fragment thereof may be disrupted. The gene of interest may be an antibody gene. The antibody gene may be one or more sets of antibody heavy-chain and light-chain genes. In the method of item 1 or 2, it is preferred that the foreign chromosome or fragment thereof contains a gene of interest and that the foreign chromosome or fragment thereof is transferred into a pluripotent cell having an endogenous disrupted gene identical with or homologous to the gene of interest and then, a chimera is produced from the pluripotent cell by using an embryo of a non-human animal in a strain deficient in an endogenous gene identical with or homologous to the gene of interest. The non-human animal in a strain deficient in an endogenous gene identical with or homologous to the gene of interest can be produced by homologous recombination in gene targeting. Preferably, the chimeric non-human animal retains the foreign chromosome(s) or fragment(s) thereof, expresses the gene(s) on the foreign chromosome(s) or fragment(s) thereof, and can transmit the foreign chromosome(s) or fragment(s) thereof to its progeny. The chimeric non-human animal is preferably a mammal, more preferably a mouse.

Please replace the paragraph that bridges pages 20 and 21 with the following paragraph:

The non-human animal of item 12 preferably retains at least one human antibody gene of at least 1 Mb and expresses the gene. The human antibody gene may be a human heavy-chain gene, a human light-chain κ gene, a human light-chain λ gene, or a combination thereof. The non-human animal of item 12 may be deficient in a non-human animal antibody gene identical with or homologous to the human antibody gene. The deficiency of non-human animal antibody gene may identical with or homologous to the human antibody gene be caused by disrupting the non-human animal antibody gene by homologous recombination.

Please replace the paragraph that bridges pages 22 and 23 with the following paragraph:

In the method of item 19, one allele of the endogenous gene in the pluripotent cell may be disrupted by homologous recombination using a drug-resistant marker gene and the other allele of the endogenous gene may be disrupted by another homologous recombination using a drug-resistant marker gene. The same drug-resistant marker gene may be used in the two homologous recombinations for disrupting one and the other alleles of the endogenous gene. Alternatively, different drug-resistant marker genes may be used in the two homologous recombinations.

Please replace the paragraph which begins 6 lines from the top of page 26 with the following paragraph:

The present invention also provides a chimeric non-human animal retaining a foreign chromosome(s) or a fragment(s) thereof and expressing the gene(s) on the foreign chromosome(s) or fragment(s) thereof, which is obtainable by one of the aforementioned methods of producing a chimeric non-human animal, or its progeny. The present invention also provides a non-human animal retaining a foreign chromosome(s) or a fragment(s) thereof and expressing the gene(s) on the foreign chromosome(s) or fragment(s) thereof which is obtainable by mating between the chimeric non-human animals or its progenies, or its progeny, retaining a foreign chromosome(s) or a fragment(s) thereof and expressing the gene(s) on the foreign chromosome(s) or fragment(s) thereof. The present invention further provides a tissue from the aforementioned chimeric non-human animal or its progeny, or the aforementioned non-human animal or its progeny. The present invention still more provides

a cell from the aforementioned chimeric non-human animal or its progeny, or the aforementioned non-human animal or its progeny. The cell may be a B cell.

Please replace the paragraph that bridges pages 27 and 28 with the following paragraph:

Moreover, the present invention provides a method of producing a biologically active substance, which comprises expressing a gene(s) on a foreign chromosome(s) or a fragment in a offspring or a tissue and a cell thereof, wherein the offspring is produced by mating the chimeric non-human animal or its progeny, or the non-human animal or its progeny retaining the foreign chromosome(s) or fragment(s) thereof and expressing the gene(s) on the foreign chromosome(s) or fragment(s) thereof with a non-human animal in a strain deficient in a gene identical with or homologous to said genes, and expressing the gene(s) on the foreign chromosome(s) or fragment(s) thereof, and recovering the biologically active substance as the expression product.

Please replace the paragraph on page 49 describing Fig. 16 with the following paragraph:

Fig. 16 is a photograph of the results of FISH analysis [[of]] showing morphology in a mouse ES cell clone (TT2 cell clone PG15) retaining partial fragments of human chromosomes #2 and 14.

Please replace the paragraph on page 50 describing Fig. 23 with the following paragraph:

Fig. 23 shows the structure of LoxP-pstNEO pLoxP-STneo plasmid DNA.

Please replace the paragraph on page 50 describing Fig. 26 with the following paragraph:

Fig. 26 shows the structures of a mouse antibody heavy-chain targeting vector and a probe for use in the Southern blotting blot analysis of genomic DNA from transformant TT2F cells, as well as a DNA fragment to be detected in homologous recombinants.

Please replace the paragraph on page 50 describing Fig. 27 with the following paragraph:

Fig. 27 shows the structures of a mouse antibody light-chain κ targeting vector and a probe for use in the Southern blotting blot analysis of genomic DNA from transformant TT2F cells, as well as a DNA fragment to be detected in homologous recombinants.

Please replace the paragraph on page 51 describing Fig. 34 with the following paragraph:

Fig. 34 is a photograph of the result of FISH analysis of an antibody heavy- and light-chains deficient mouse ES cell clone retaining ~~partial~~ fragments of human chromosomes #2 and #14.

Please replace the paragraph on page 51 describing Fig. 36 with the following paragraph:

Fig. 36 is a photograph showing the result of FISH analysis of a mouse A9 cell containing fragments of human chromosome #14 (human centromere sequence probe).

Please replace the paragraph which begins on page 51 describing Fig. 37 with the following paragraph:

Fig. 37 is a photograph showing the result of FISH analysis of a mouse A9 cell containing fragments of human chromosome #14 (human chromosome-specific probe).

Please replace the paragraph on page 52 describing Fig. 42 with the following paragraph:

Fig. 42 shows the results of analysis of complete human antibody-producing mouse strains established by mating.

Please replace the paragraph bridging pages 60 and 61 with the following paragraph:

3) A targeting vector retaining a human telomere sequence is constructed and the telomere sequence is inserted in the cell in a cell retaining a human chromosome at a desired

site on a chromosome by homologous recombination in a cell retaining a human chromosome the cell. After a clone into which the telomere sequence has been inserted is obtained, a mutant having deletion caused by the telomere truncation is obtained. See Itzhaki et al., Nature Genet., 2, 283-287, 1992 and Brown et al., P. N. A. S., 93:7125, 1996. As a host into which a targeting vector is to be introduced, a cell allowing for high-frequency homologous recombination such as a chicken DT40 cell (Dieken et al., supra) may also be used. Telomere truncation of human chromosomes in a chicken DT40 cell is first disclosed in the present invention. Brown (supra) discloses that a vector was inserted into a repeat sequence on a chromosome. However, no specific site can be targeted. Itzhaki et al. discloses that tumor cells, i.e., 12000 cells of cell line HT1080 into which a telomere sequence was introduced were analyzed and 8 homologous recombinants were obtained. They found that out of the 8 cells, only one caused deletion by insertion of the telomere sequence. For some kinds of cells, results were reported that no mutant having truncation was obtained by insertion of a telomere sequence into some kinds of cells (Barnett et al., Nucleic Acids Res., 21:27, 1993). In spite of this report, the inventors believed that it was necessary to increase the absolute number of homologous recombinants in order to obtain mutants having truncation and made an attempt to perform telomere truncation using a chicken DT40 cell as a host. As a result, it was surprisingly found that truncation occurred in all of the 8 homologous recombinants obtained.

Please replace the paragraph bridging pages 85 and 86 with the following paragraph:

The chimeric mice or their progenies that retain human chromosomes #2, 14 and/or 22 (or fragments thereof) which can be produced by the method of the present invention can retain the greater part of the functional sequences of respective genes for human antibody heavy chain on chromosome #14, light chainκ on chromosome #2 and light chain λ on chromosome #22. Hence, they can produce a wide repertory of antibodies which are more similar to human antibody repertory, compared with known transgenic mice into which parts of human antibody gene have been transferred by using yeast artificial chromosomes and the like (Green et al., Nature Genetics, 7, 13-, 1994; Lonberg et al., Nature, 368, 856-, 1994). Also, the chimeric mice and their progenies retaining two human chromosomes (or fragments) of #2+#14, #22+#14 or other combination and the mice and their progenies retaining three human chromosomes (or fragments) of #2+#14+#22 or other combination

which are obtainable by mating said chimeric mice and their progenies retaining two human chromosomes (or fragments), as produced by the method of the invention, can produce complete human antibodies both heavy- and light-chains of which are derived from human. These mice can recognize human-derived antigens as foreign substances to cause an immunoreaction with the antigens, thereby producing antigen-specific human antibodies. These properties can be utilized to produce human monoclonal and polyclonal antibodies for therapeutic treatments (Green et al, supra; Longberg et al., supra). On the other hand, in order to obtain a human antibody having high affinity for a particular antigen more efficiently, it is desirable to produce a mouse which produces a human antibody but not a mouse antibody (Green et al., supra; Lonberg et al., supra). In the present invention, this is achieved typically by the following Method A or B ~~using known techniques~~.

Please replace the first full paragraph on page 88 with the following paragraph:

3. An enzyme gene (e.g., a Cre recombinase gene (Sauer et al., supra)) which causes a site-directed recombination between the recombination sequences inserted at both the ends of the drug-resistance gene in step 1 above is transiently transferred into the mouse ES cells from step 2 above in which both alleles of antibody heavy-chain genes were disrupted. Then, drug-sensitive clones are selected in which the drug-resistance genes inserted at the sites of both heavy-chain genes were deleted as a result of recombination between the loxP sequences [Seiji Takatsu et al., "Experimental Medicine (extra number): Basic Technologies in Immunological Researches", p. 255-, published by Yodosha, 1995].

Please replace the paragraph bridging pages 193-195 with the following paragraph:

In order to obtain a recombinant in which an antibody heavy-chain gene has been disrupted by homologous recombination (hereinafter, referred to as an "antibody heavy-chain homologous recombinant"), the antibody heavy-chain targeting vector prepared in Section 3, Example 48 was linearized with restriction enzyme SacII (Takara Shuzo), and transferred into mouse TT2F ES cells according to the method described by Shinichi Aizawa, "Biomanual Series 8, Gene Targeting", published by Yodosha, 1995. The TT2F cells were treated with trypsin and suspended in HBS at a concentration of $2.5 \times 10^7$ cells/ml. To the cell suspension, 5μg of DNA was added. Then, electroporation was performed with a gene pulser (Bio-Rad

Laboratories, Inc.; resistor unit not connected). A voltage of 250 V was applied at a capacitance of 960μF using an electroporation cell of 4 mm in length at room temperature. The electroporated cells were suspended in 20 ml of an ES medium and inoculated into two tissue culture plastic plates (Corning) of 100 mm into which feeder cells were seeded preliminarily. Similarly, experiments using 10 and 15 μg of DNA were also conducted. After one day, the medium was replaced with a medium containing 300μg/ml of G418 (GENETICIN; Sigma). Seven to nine days thereafter, a total of 176 colonies formed were picked up. Each colony was grown up to confluence in a 12-well plate, and then four fifths of the culture was suspended in 0.2 ml of a preservation medium [ES medium + 10% DMSO (Sigma)] and stored frozen at -80°C. The remaining one fifth was inoculated into a 12-well gelatin coated plate and cultured for 2 days. Then, genomic DNA was obtained from the cells ($10^6$-$10^7$ cells) with Puregene DNA Isolation Kit (Gentra System Co.). These genomic DNAs from G418 resistant TT2F cells were digested with restriction enzymes EcoRI and XhoI (Takara Shuzo) and separated by agarose gel electrophoresis. Then, Southern blotting was performed to detect homologous recombinants with the probe described in Section 3, Example 48. As a result, 3 clones out of the 176 clones were homologous recombinants. The results of Southern blot analysis of wild-type TT2F cells and homologous recombinants #131 and #141 are shown in the left-side three lanes in Fig. 28. In wild-type TT2F cells, two bands (a and b) [[are]] <u>were</u> detected which [[were]] <u>had been</u> obtained by the EcoRI and XhoI digestion. In the homologous recombinants, it is expected that one of these bands disappears and that a new band (c) will appear at the lower part of the lane. Actually, band (a) has disappeared in #131 and #141 in Fig. 28 and a new band (c) has appeared. The size of DNA is shown at the left side of the Figure. These results show that one allele of an antibody heavy-chain gene in these recombinant clones has been disrupted by homologous recombination.

Please replace the paragraph bridging pages 197 and 198 with the following paragraph:

It has been reported that a clone in which both alleles are disrupted can be obtained by disrupting one allele by insertion of a G418 resistance gene, culturing an ES cell clone in a medium with an increased G418 concentration and screening the resultant high concentration

G418 resistant clones (Shinichi Aizawa, "Biomanual Series 8, Gene Targeting", published by Yodosha, 1995). Based on this technique, the inventors have conducted the following experiments in order to obtain both alleles-disrupted clones from the TT2F antibody heavy-chain homologous recombinants #131 and #141. First, in order to determine the lethal concentration of G418 for both #131 and #141 clones, each clone was inoculated into ten 35 mm plates at a rate of about 100 cells per plate (in this Example, G418 resistant primary culture cells which were purchased from Lifetech Oriental and were not treated with mitomycin were used as feeder cells)(see Example 9). The cells were cultured in an ES medium containing 0, 0.5, 1, 2, 3, 5, 8, 10, 15 and 20 mg/ml of G418 (GENETICIN, Sigma) for 10 days. As a result, definite colonies were observed at a concentration of up to 3 mg/ml, but no colony formation was observed at 5 mg/ml. Based on these results, the minimum lethal concentration was decided to be 5 mg/ml. Then, high concentration G418 resistant clones were selected at concentrations of 4, 5, 6, 7 and 8 mg/ml. For each of #131 and #141, cells were inoculated into ten 100 mm plates at a rate of about $10^6$ cells per plate and cultured in an ES medium containing G418 at each of the concentrations described above (5 grades; two plates for each concentration). Twelve days after the start of culture, definite colonies (#131: 12 clones; #141: 10 clones) were picked up from plates of 7 mg/ml and 8 mg/ml in G418 concentration. These clones were stored frozen and genomic DNA was prepared by the same procedures as in Example 49. The genomic DNAs from these high concentration G418 resistant clones were digested with restriction enzymes EcoRI and XhoI (Takara Shuzo) and separated by agarose gel electrophoresis. Then, Southern blotting was performed to detect with the probe from Section 3, Example 48 those clones in which both alleles have been disrupted. As a result, one clone derived from #131 (#131-3) was found to be both alleles-distrupted clone. The results of Southern blot analysis of 6 clones derived from #131 are shown in Fig. 28. In wild-type TT2F cells, two wild-type bands (a, b) [[are]] were detected after the EcoRI and XhoI digestion. In one allele homologous recombinants (#131, #141), the upper band (a) [[has]] was disappeared and a new band (c) [[has]] was appeared (Example 49). Furthermore, it is expected that due to the disruption of both alleles, another wild-type band (b) disappears and that the disruption-type band (c) remains alone.

Please replace the paragraph bridging pages 199 and 200 with the following paragraph:

The G418 resistance marker gene in the antibody heavy-chain both alleles-disrupted clone (high concentration G418 resistant clone #131-3) from Example 51 was removed by the following procedures. An expression vector, pBS185 (BRL), containing Cre recombinase gene which causes a site-directed recombination between the two LoxP sequences inserted at both the ends of the G418 resistance gene was transferred into #131-3 clone according to the methods described in Shinichi Aizawa, "Biomanual Series 8, Gene Targeting", published by Yodosha, 1995 and Seiji Takatsu et al., "Experimental Medicine (extra number): Basic Technologies in Immunological Researches", p. 255-, published by Yodosha, 1995). Briefly, #131-3 cells were treated with trypsin and suspended in HBS to give a concentration of $2.5x10^7$ cells/ml. To the cell suspension, 30 μg of pBS185 DNA was added. Then, electroporation was performed with a gene pulser (Bio-Rad Laboratories, Inc.; resistor unit not connected). A voltage of 250 V was applied at a capacitance of 960 μF using an electroporation cell of 4 mm in length (see Example 1) (165-2088, Bio-Rad Laboratories, Inc.). The electroporated cells were suspended in 5 ml of an ES medium and inoculated into a tissue culture plastic plate (Corning) of 60 mm in which feeder cells were seeded preliminarily. After two days, the cells were treated with trypsin and reinoculated into three 100 mm plates (preliminarily seeded with feeder cells) such that the three plates have 100, 200 and 300 cells, respectively. A similar experiment was also conducted under the same conditions except that the setting of the gene pulser was changed (resistor unit connected; resistance value infinite). After seven days, a total of 96 colonies formed were picked up and treated with trypsin. Then, the colonies were divided into two groups; one was inoculated into a 48-well plate preliminarily seeded with feeder cells and the other was inoculated into a 48-well plate coated with gelatin alone. The latter was cultured in a medium containing 300μg/ml of G418 (GENETICIN, Sigma) for three days. Then, G418 resistance was judged from the survival ratio. As a result, 6 clones died in the presence of G418. These G418 sensitive clones were grown to confluence in 35 mm plates, and four fifths of the resultant culture was suspended in 0.5 ml of a preservation medium [ES medium + 10% DMSO (Sigma)] and stored frozen at -80°C. The remaining one fifth was inoculated into a 12-well gelatin coated plate and cultured for two days. Thereafter, genomic DNA was prepared by the same procedures as in Example 2 from $10^6$ to $10^7$ cells using Puregene DNA Isolation Kit(Gentra System).

Please replace the paragraph bridging pages 200 and 201 with the following paragraph:

These genomic DNAs from G418 sensitive TT2F clones were digested with restriction enzyme EcoRI (Takara Shuzo) and separated by agarose gel electrophoresis. Then, Southern blotting was performed to confirm the removal of the G418 resistance gene using a 3.2 kb XhoI fragment (Probe A) from G418 resistance gene-containing pSTneoB. As a result, bands observed in #131-3 clone which hybridize with Probe A were not detected at all in the sensitive clones. From these results, it was confirmed that the G418 resistance marker gene had been surely removed in the G418 sensitive clones obtained. Additionally, as a result of Southern blot analysis performed in the same manner using Probe B obtained by digesting pBS185 DNA with EcoRI, no specific band which hybridizes with Probe B was detected in these G418 sensitive clones. Thus, it is believed that Cre recombinase-containing pBS185 is not inserted into the chromosomes of the sensitive clones. In other words, these sensitive clones can be transformed with the vector for knocking out an antibody light-chain (vector having a loxP sequence at both the ends of a G418 resistance gene) described in Section 4, Example 48. Chimeric mice were produced from the G418 sensitive clone #131-3-5 in the same manner as in Example 40. As a result, chimeric mice exhibiting 100% contribution to coat color were obtained.

Please replace the paragraph which begins at the top of page 205 with the following paragraph:

A homologous recombinant, which has further disruption in an antibody light-chain gene in the antibody heavy-chain-deficient homozygote TT2F cell clone (G418 sensitive) obtained in Example 52 is produced by the following procedures. Briefly, the antibody light-chain targeting vector prepared in Section 4, Example 48 is linearized with restriction enzyme KpnI (Takara Shuzo), and transferred into the above TT2F cell clone (G418 sensitive) according to the method described in Shinichi Aizawa, "Biomanual Series 8: Gene Targeting", published by Yodosha, 1995, and in the same manner as in Example 49. Briefly, the cells are suspended in HBS to obtain a suspension at 2.5 x $10^7$ cells/ml, and then 5 μg of DNA is added to 0.5 ml of the cell suspension. A voltage of 250 V was applied to electroporation cells at 960 F. After 7-9 days, colonies formed [[are]] were picked up. They

[[are]] were stored frozen and genomic DNA [[is]] was prepared in the same manner as in Example 49. Genomic DNAs from G418 resistant clones [[are]] were digested with restriction enzymes EcoRI and NotI (Takara Shuzo) and separated by agarose gel electrophoresis. Then, Southern blot analysis is performed to detect homologous recombinants with the probe described in Section 4, Example 48. The results are shown in Figure 29. In the parent clone, the antibody heavy chain-deficient homozygote TT2F cell, a band (a) was detected after EcoRI digestion. It is expected that the band (a) and a new band (b) below the band (a) may be observed in the homologous recombinant. In the Figure, the band (b) was observed in the transformants 2 and 5. And in the left side of the Figure, the size of DNA is indicated. Therefore, one allele of the antibody light chain gene in these clones was disrupted by homologous recombination. Twenty-eight clones were homologous recombinants among 120 clones analyzed. It suggests that these clones do not exhibit any change in their growth rate and morphology compared to the TT2F clone without gene disruption in the conventional culture conditions, and have a capability to form a chimeric mouse.

Please replace the paragraph bridging pages 205 and 206 with the following paragraph:

A clone in which both alleles of a light-chain gene are disrupted is prepared from the TT2F antibody light-chain homologous recombinant (and antibody heavy-chain-deficient homozygote) clone from Example 58 by the procedures described below. Briefly, a high concentration G418 resistant clone [[is]] was prepared [[and]] in the same manner as in Example 51. The clones were cultured in the presence of 9 to 14 mg/ml of G418 for 5 to 7 days, and then cultured in the presence of 4 mg/ml of G418. The colonies grown after 10 to 13 days from the first day of culturing were picked up, stored frozen, and DNA [[is]] was prepared in the same manner as in Example 51. Genomic DNA from the high concentration G418 resistant clone [[is]] was digested with restriction enzymes EcoRI and NotI (Takara Shuzo) and separated by agarose gel electrophoresis. Then, Southern blot analysis [[is]] was performed to detect those clones in which both alleles have been disrupted, with the probe from Section 4, Example 48. The results of Southern blot analysis are shown in Figure 32. It is expected that the band (a) shown in Figure 32 may be disappeared and only the band (b) can be observed in the double knock-out clone. In the Figure 32, the bands are not observed

in the high concentration G418 resistant clones 2, 3 and 8. Therefore, both alleles of the antibody light chain gene in these clones were disrupted by homologous recombination. The high concentration resistant clones obtained from 3 independent clones of one allele-disrupted clones were analyzed, and 36, 2 and 1 clones having the both alleles-disrupted antibody light chain gene were obtained among 59, 43 and 49 clones, respectively. It suggests that these clones do not exhibit any change in their growth rate and morphology compared to the TT2F clone without gene disruption in the conventional culture conditions, and have a capability to form a chimeric mouse.

Please replace the paragraph bridging pages 206 and 207 with the following paragraph:

The G418 resistance marker gene in the antibody light-chain both alleles-disrupted clone (high concentration G418 resistant clone) obtained in Example 59 [[is]] was removed by the same procedures as in Example 52. Briefly, an expression vector, pBS185 (BRL), containing Cre recombinase gene which causes a site-directed recombination between the two loxP sequences inserted at both the ends of the G418 resistance gene (Section 1, Example 48) was transferred into the above clone according to the method described in Example 52. The resultant G418 sensitive clones [[are]] were grown to confluence in 35 mm plates, and 4/5 of the resultant culture was suspended in 0.5 ml of a preservation medium [ES medium + 10% DMSO (Sigma)] and stored frozen at -80°C by the same procedures as in Example 52. The remaining 1/5 was inoculated into a 12-well gelatin coated plate. After cultivation for two days, genomic DNA [[is]] was prepared by the method described in Example 2 from $10^6$ to $10^7$ cells using Puregene DNA Isolation Kit(Gentra System). These genomic DNAs from G418 sensitive TT2F clones [[are]] were digested with restriction enzyme EcoRI (Takara Shuzo) and separated by agarose gel electrophoresis. Then, Southern blotting [[is]] was performed to confirm the removal of the G418 resistance gene using a 3.2 kb XhoI fragment from G418 resistance gene-containing pSTneoB as a probe.